"Cytotoxic and anti-oxidant activity of petroleum ether fraction of *Mentha arvensis leaf extract*"

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EAST WEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF PHARMACY

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Declaration by the Research Candidate

I, Afruja Sultana, hereby declare that the dissertation entitled **"Cytotoxic and anti-oxidant activity of petroleum ether fraction of *Mentha arvensis* leaf extract"** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2015-2016, under the supervision and guidance of Abdullah-Al-Faysal, Senior Lecturer, Department of Pharmacy, East West University and the thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled **"Cytotoxic and anti-oxidant activity of petroleum ether fraction of *Mentha arvensis* leaf extract"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by Afruja Sultana, ID# 2011-3-70-002 in 2016, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Dedication

This Research Paper is dedicated to my beloved Parents and my friends.

Abstract

The plant *Mentha arvensis* has been used for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases like asthma, piles, diarrhea etc. The aim of the present study was to evaluate the cytotoxicity activity, and antioxidant activity of pet-ether extract of *Mentha arvensis*. The powdered leaves of *Mentha arvensis* were extracted with methanol and then partitioned with pet-ether, ethyl acetate and dichloromethane consecutively. The pet-ether fraction was used to evaluate cytotoxic, and antioxidant activities. The cytoxic activity was measured by brine shrimp lethality bioassay. Pet-ether fraction showed cytotoxic activity with LC_{50} value 17.79µg/ml in brine shrimp lethality test. The fraction contained 12.315mgAAE/g of total phenolic content and 56mg AAE/g total flavonoid content and 27.5mg AAE/g total reducing power content. The results of study clearly indicate the presence of cytotoxic, and antioxidant properties of pet-ether extract. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key word: *Mentha arvensis,* Cytotoxic test, Anti-oxidant test, Phenolic content, Flavonoid content, Reducing power assay

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List of Abbreviation

Meaning of abbreviated form	Abbreviated form
Ascorbic Acid Equivalent	AAE
Dimethyl Sulfoxide	DMSO
Gram	g or gm
Hour	hr
Lethal concentration required to kill 50% of the sample population	LC50
Microgram	µg
Micro liter	µl
Milligram	mg
Milliliter	ml
Petroleum ether	Pet-ether
Ultraviolet	UV
World Health Organization	WHO

Chapter 1: Introduction

1.1 Medicinal plant

Medicinal plants are plants which have a recognized medical use. It's containing essential bioactive ingredients are used to cure disease or disorder since time immortal. One of the aims of medicinal plant research is the isolation and identification of markers/ bioactive compounds. Isolation of the markers compounds and bioactive plant constituents has always been a challenging task for the researchers. Separation of these components from the medicinal plants includes the use of combination of chromatographic techniques such as column chromatography,preparative thin layer chromatography, preparative high performance liquid chromatography, droplet counter current chromatography, centrifugal thin layer chromatography, etc. Centrifugal thin layer chromatography which makes use of centrifugal force for separation of multi-component system offers extensive platform for the isolation of phytoconstituents from medicinal plants. This review focuses on basic principle, instrumentation and advantages of centrifugal thin layer chromatography (Agrawal& Desai, 2015).

1.1.1 Definition

The plants having therapeutic or medicinal effects are called medicinal plants. The term 'medicine' can be referred to a preparation or as compound containing one or more drugs or therapeutic agents which are used in the treatment, cure or mitigation of various diseases and external or internal injuries of man and other animals (Ghani, 1998).

Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: " A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesizing of useful drugs" (Sofowara, 1982).

1.1.2 History of Plants in medicine

The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies. The 'Pen Tsao' contains thousands of herbal cures attributed to Shen-nung, China's legendary emperor who lived 4500 years ago. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection

of Hindu sacred verses. The Badianus Manuscript is an illustrated document that reports the traditional medical knowledge of the Aztecs. Western medicine can be traced back to the Greek physician Hippocrates, who believed that disease had natural causes and used various herbal remedies in his treatments.

Early Roman writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in De Materia Medica. Many of the herbal remedies used by the Greeks and Romans were effective treatments that have become incorporated into modern medicine (e.g., willow bark tea, the precursor to aspirin). Dioscorides' work remained the standard medical reference in most of Europe for the next 1500 years (Bryan *et al*., 1989).

The beginning of the Renaissance saw a revival of herbalism in the identification of medicinally useful plants. This coupled with the invention of the printing press in 1450 ushered in the Age of Herbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses of plants, but also included much misinformation and superstition. The Doctrine of Signatures, for example, held that the medicinal use of plants could be ascertained by recognizing features of the plant that corresponded to human anatomy. For example, the red juice of bloodwort suggests that it should be used for blood disorders; the lobed appearance of liverworts suggests that it should be used to treat liver complaints; the "humanoid" form of mandrake root suggests that is should be used to promote male virility and ensure conception (Ghani, 1998).

Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally

isolated from plants. While Western medicine strayed away from herbalism, 75% to 90% of the rural population of the rest world still relies on herbal medicine as their only health care (Levetin and Mahon, 2003).

In many village marketplaces, medicinal herbs are sold alongside vegetables and other wares. The People's Republic of China is the leading country for incorporating traditional herbalmedicine into a modern health care system; the result is a blend of herbal medicine, acupuncture, and Western medicine. Plantations exist in China for the cultivation of medicinal plants, and thousands of species are thus available for the Chinese herbalist; prescriptions are filled with measured amounts of specific herbs rather than with pills or ointments. In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine, with its Muslim and Greek roots, is another widely practiced herbal tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices (Levetin and Mahon, 2003).

1.1.3 Medicinal Plants as Drugs

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal and human body are generally designated as medicinal plants (Ghani, 1998).

According to the World Health Organization (WHO),

- A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. (Sofowara,1982).

When a plant is designated as medicinal, it is implied that the plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation.

- Medicinal plants may therefore be defined as a group of plants that possess some

special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes‖. Many of the plants could be used as stimulants, poisons, hallucinogens or as medicine because of the presence of unique or rich biological-active plant chemicals (i.e. Chemical compounds that have a biological effect on another organism (Hamburger & Hostettmann, 1991).

1.1.4 Characteristics of Medicinal plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

- Support of official medicine- In the treatment of complex cases like cancer cancer diseases the components of the plants proved to be very effective.

- Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Hasan, 2012).

1.1.5 Importance's of studying medicinal plant

- A future medicine bank to discover. There are approximately half a million plants with flowers, most of which have not been investigated and which principles could be decisive in the treatment of present or future diseases.
- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.
- Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.

- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.

1.1.6 Use of Medicinal Plant in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (IUCN, 2003). The number of medicinal plants included in "Materia medica" of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants, majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (Kritikar and Basu, 1980).

Bioactive compounds deposited in medicinal plants can serve as important raw materials for pharmaceutical manufacturing. Therefore, well-judged and scientific investigation of this wealth can significantly contribute to the public health. Again, it was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries. Thus, being available commodity of commerce, a country can also earn a good amount of foreign currency by exporting this natural wealth to other countries (Chopra *et al*,. 1982).

1.2 Description of the *Mentha arvensis*

1.2.1 Scientific name

- *Calamintha arvensis* (L.) Garsault [Invalid]
- *Mentha arvensis* Linn.
- *Mentha parietariifolia* Becker ex Steud.

1.2.2 Common name

- Ablebana (If.)
- Herba buena (Tag.)
- Hilbas (Tag.)
- Karabo (Surigao del Norte)
- Menta
- Brook mint (Engl.)
- Corn mint (Engl.)
- Field mint (Engl.)
- Marsh mint (English)
- Pepper mint (Engl.)
- Tule mind (Engl.)
- Wild mint (Engl.) (Hasan, 2012)

1.2.3 Local name

- Pudina pata.

1.2.4 General Species Description

Mentha arvensis is an erect herbacious perennial plant that grows 20 - 80 cm tall on square, hairy stems. It is rhizomatous and has opposite leaves that are attached to the stem with very short stalks. It has a very fragrant minty aroma.

Figure 1.1: *Mentha arvensis*plant.

1.2.4.1 Leaves

The leaves are 2 - 8 cm long and 6 - 40 mm wide. They are lance to oval shaped, taper to a point, and have sharply toothed margins.

Figure 1.2: Leaf of *Mentha arvensis*

1.2.4.2 Inflorescence/Flowers

The flowers are whorled around the upper leaf axils. They are 4 - 7 mm long, 2 lipped, irregular, and range from white to pink to violet. They have longer stamens than petals. They bloom from June to August.

Figure 1.3: Flower of *Mentha arvensis*

1.2.4.3 Fruits

The fruits are brown nutlets. There are four per flower and they enlarge and remain on the plant into the winter. Flower whorls c 10-18 mm diameter.

Figure 1.4: Fruits of *Mentha arvensis*

1.2.4.4 Habitat

Mentha arvensis grows in moist places; along shorelines and streambanks, in wet meadows, prairies, and ditches, and in open spaces in shrub swamps. It is found from low to moderate elevations. Juncus species and Veronica species are commonly found with the Menthas.

1.2.4.5 Range

Mentha arvensis is found all the way around the north pole, extending as far south as New Mexico. It is the only native mint species here in the northwest, and is also the most common. It is found extensively west of the Cascades.

1.2.4.6 Similar Species

Lycopus americanus and *Lycopus uniflorus* both have square stems. They, however, do not have a strong odor. They have fewer, smaller flowers, and the petals are smaller than the stigmas. *Mentha spicata* and *Mentha piperita* can be distinguished by their terminal inflorescence.

1.2.4.7 Ecological Value

The fruits of *Mentha arvensis* are widely used as a food source for small animals.

1.2.4.8 Human Value

Native Americans used field mint tea for colds. Today we still use it for medicinal purposes. It is widely used for upset stomachs, gas, and sore throats. It can also be used for culinary purposes, and in hygeine products. (Web.pdx.edu, 2016)

1.2.5 Taxonomic position

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Lamiales
Family	: Lamiaceae – Mint family
Genus	: *Mentha* L. – mint P

1.3 Use of *Mentha arvensis*

1.3.1 Nutritional

- ✓ Cultivated as a spice for cooking.
- ✓ Leaves used for tea.
- ✓ Used in salads to provide flavor.
- ✓ Used as a flavoring in confections and dentifrices.

1.3.2 Folkloric

- ➢ One of the oldest household remedies known.
- ➢ In the Philippines, tops and leaves are considered carminative; when bruised used as antidote to stings of poisonous insects.
- ➢ Mint is used in neuralgic affections, renal and vesical calculus.
- ➢ Used for stomach weakness and diarrhea.

- Decoction and infusion of leaves and stems used for fever, stomach aches, dysmenorrhea, and diuresis.
- Pounded leaves for insect bites, fevers, toothaches, headaches.
- Crushed fresh plants or leaves are sniffed for dizziness.
- Powdered dried plant as dentifrice.
- Crushed leaves are applied on the forehead and temples for headaches.
- For toothaches:
  - Wet a small piece of cotton with juice expressed from crushed leaves; apply this impregnated cotton bud to the tooth.
  - Boil 6 tbsp. of leaves in two glasses of water for 15 minutes; strain and cool. Divide the decoction into 2 parts and take every 3 to 4 hours.
- Plant used as emmenagogue; also used in jaundice.
- Dried plant used as dentifrice.
- Leaves and stems used as carminative, antispasmodic, and sudorific.
- Infusion of leaves used for indigestion, rheumatic pans, arthritis and inflamed joints.
- For coughs, boil 6 tbsp of chopped leaves in 2 glasses of water for 15 mins; cool and strain. Divide the decoction into three parts; take 1 part 3 times a day.
- Diluted essential oil used as wash for skin irritations, burns, pruritus, scabies, ringworm and as mosquito repellent.
- For arthritis, warm fresh leaves over low flame; then pound. Apply pounded leaves while warm on the painful joints or muscles.
- As mouthwash, soak 2 tbsp chopped leaves in 1 glass of hot water for 30 minutes; strain. Use the infusion as mouthwash.

1.3.3 Others

- ✓ Peppermint oil is often used in pharmaceutical preparations to subdue unpleasant medicinal smells.
- ✓ Menthol derived from the essential oil is used in pharmaceutical, perfumery and food industries.

1.4 Studies of *Mentha arvensis*

- **Radioprotective/Leaves**: Study of mint extract on mice showed benefit with pretreatment of mice with reduction in the severity of symptoms of radiation sickness and mortality.
- **Anti-candida**: A study of essential oils and ethanolic extracts of leaves/roots of 35 medicinal plants in Brazil screened for anti-Candida activity. *Mentha arvensis* was one of 13 essential oils that showed anti-candidal activity.
- **Anti-fertility/Male Contraceptive:** A study of the ether extract of MA on male mice showed reduction of number of offspring, with decrease in testes weight, sperm count and motility, among others. Results suggest that the ether extract of MA possess reversible anti-fertility properties.
- **Reversible Male Contraceptive Effect:** Study of aqueous extract solution in male mice caused inhibition of fertility while maintaining normal sexual behavior. All induced effects returned to normalcy within 30 days of withdrawal of 60-day treatment.
- **Post-coital Anti-fertility Effect**: A study on the uterotonic fraction of MA caused significant interruption in pregnancy in rats, pronounced in the post-implantation period.
- **Antibiotic Resistance-Modifying**: A report on the ethanol extract of MA showed a potentiating effect of the extract on Gentamicin and presents a potential against bacterial resistance to antibiotics.
- **Potentiatiing Effect with Chlorpromazine against Bacterial Resistance:** Study showed extracts of M arvensis could be used as a source of plant-derived natural products with resistance-modifying activity, such as in the case of amino glycosides - a new weapon against bacterial resistance to antibiotics, as with chlorpromazine.
- **Anti-Gastric Ulcer**: Study of various extracts of *Mentha arvensis* showed a protective effect against acid secretion and gastric ulcers in ibuprofen plus pyloric ligation-induced and 90% ethanol-induced ulcer models.
- **Herbal Liniment / Analgesic**: M arvensis provides potent analgesic action and is used externally in rheumatism, neuralgia and headaches. In an herbal liniment where it was combined with four other medicinal plants, the liniment was found

effective in ligament or muscle injury pain (sprains, strains, spasms, tennis elbow, etc), less so in osteoarthritis of the joint and periarthritis of the shoulder. No adverse reactions were reported. Efficacy was noted better in synergism with oral or parenteral analgesics.

- **Volatile Constituents / Menthol:** Study showed the shoot leaf gave the highest yield of oil, 0.62%; while the stems had negligible yield. Menthol was the major component of all the oils. Other oils identified were: B-caryophyllene oxide, a-phellandrene, terpinolene, limonene, menthone and pulegone.
- **Linarin / Anti-Acetylcholinesterase:** Flowers extract of M arvensis yielded linarin (acacetin-7-0-b-D-rutinoside), with selective dose-dependent inhibitory effect on acetylcholinesterase.
- **Anti-Allergic / Anti-Inflammatory:** Study on anti-allergic activity using a histamine inhibitory assay showed the ethanol extracts of leaf and root markedly inhibited the release of histamine from mast cells. On anti-inflammatory testing using a histamine-induced paw edema model, all extracts showed anti-inflammatory effect suggesting the presence of compounds capable of inhibiting histamine release from the mast cells and/or block histamine receptors.
- **Effect on Haloperidol-Induced Catalepsy:** Study in mice suggested *Mentha arvensis* significantly reduced oxidative stress and cataleptic score induced by haloperidol. Results suggest it can be used to prevent the drug-induced pyramidal side effects.
- **Antifungal Activity / Leaves / Study Against Oral Pathogens:** Study evaluated hydroalcoholic extracts for antimicrobial activity against oral pathogens: Streptococcus mutans, S. sobrinus and Candida albicans. Results showed antifungal activity against C. albicans and a potential use for human antifungal use. Results showed no antibacterial effect.
- **Hepatoprotective / CCl4-Induced Liver Damage:** Study evaluated various extracts of leaves against carbon tetrachloride induced liver damage in rats. Results showed a hepatoprotective effect with significant reductions of liver enzymes almost comparable to silymarin. Hepatoprotection was confirmed by histopathological examination. Phytochemical screening yielded flavonoids, steroids, triterpenoids, alkaloids, glycosides, carbohydrates, tannins, phenolic compounds.

- **Antioxidant:** Study evaluated the antioxidant activity of an ethanol extract of leaves of *M. arvensis* through various assays:TBAR, DPPH, NO radical scavenging, superoxide radical scavenging and phosphomolybdonum method. Results showed significant dose-dependent antioxidanat activity in all the assays. A methanol extract of roots exhibited good antioxidant potential using DPPH, reducing power, metal chelating, nitrous oxide scanging and hydrogen peroxide scavenging assays.
- **Anthelmintic / Leaves:** Study evaluated the anthelmintic activity of leaves of *M. arvensis* against Ascardia galli which resembles the nematode Ascaris lumbricoides. Results showed a petroleum ether extract with maximum anthelmintic activity probably through both blocking of energy metabolism and worm paralysis.
- **Anticargiogenic:** Study evaluated the efficacy of crude extract and solvents of *M. arvensis* against human cariogenic pathogens Streptococcus mutans, Streptococcus sanguinis, Staphylococcus aureus, and Lactobacillus casei isolated from patients with dental disease. Analysis for secondary metabolites yielded alkaloids, tannins flavonols, steroids, xanthones and glycosides. Results showed significant amounts of phytochemicals with antioxidative properties which could be responsible for its antimicrobial property.
- **Antidepressant / Antioxidant:** Study of aqueous extracts in Swiss albino mice showed significant in vitro antioxidant (DPPH, NO, and hydroxyl radical scavenging assays) and antidepressant (Tail suspension and Forced swim tests) activity.
- **Anticancer / Growth Suppression and Apoptosis:** Study evaluated *Mentha arvensis* for in vitro cytotoxicity against human cancer cell line (Hep G2 cell line). Results showed MA significantly suppresses growth and induces apoptosis.
- **Comparative Antioxidant Study:** Study evaluated powdered plants extracts of M. arvensis, Allium porrum and Elettaria cardamomum for antioxidant activity using free radical scavenging assays. *Mentha arvensis* exhibited the maximum content of phenols and flavonoid compounds, and greatest antioxidant profile.
- **Nanoparticles / Leaves / Antioxidant Study:** Study showed *Mentha arvensis* mediated silver nanoparticle arvensis exhibited high antioxidant properties.
- **Nanoparticles / Leaves / Antioxidant Study:** Study of ethanolic extract of *M.*

arvensis in albino mice showed free radical scavenging activity by DPPH assay, antimicrobial activity against S. typhi, S. paratyphi, S. boydii, S. pyogenes and S. aureus, cytotoxic lethality against brine shrimp nauplii, and analgesic effect in acetic acid induced writhing.

- **Nephroprotective in Cisplatin Induced Toxicity:** Study of evaluated the effect of *M. arvensis* on cisplatin-induced nephrotoxicity in Sprague-Dawley rats. Results showed nephroprotective action evidenced by blood parameters and histopathological studies, and attributed to the presence of flavonoids and related compounds.
- **Thrombolytic / Cytotoxic:** Study of *Mentha arvensis*, M. spicata, and M viridis showed clot lysis activity and low cytotoxicity activity. Results suggest the plants can be incorporated as thrombolytic agents with in vivo effects to improve the atherothrombotic patients.
- **Essential Oil / Aerial Parts / Use for Reduction of Mental Stress:** Study showed the fragrant chemicals of essential oil of *M. arvensis* reduce the level of mental stress and has a potential for use in the treatment of psychophysiological disorders. (see constituents above) .
- **Absorptive Removal of Chromium Ions / *M. arvensis* Biomass:** Study investigated the binding capacity of *M. arvensis* biomass for chromium ions from aqueous synthetic effluents. Results showed MA biomass can be used for removal of toxic ions from industrial effluents. (Hasan, 2016)

1.5 Aims of the Present Study

Attempts should be continued for the evaluation of the cytotoxic, antimicrobial, and antioxidant activity of the aqueous fraction of *Mentha arvensis*leaves extract. To conduct cytotoxic investigation of aqueous extract by brine shrimp lethality bioassay. To investigate in vitro antioxidant property of aqueous extract by total Phenolic content and total Flavonoid content. *Mentha arvensis*is a very common plant which is used in our country as well as in world by a lot of people for several purposes. All the parts of this plant are used for medicinal activity. To achieve this objective, the whole work was designed in the following way:

a. Cytotoxic study with pet-ether fraction.
b. Anti-oxidant study with pet-ether fraction.

Chapter 2: Literature Review

2.1 Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of *Mentha arvensis* L. in male albino mice.

In male albino mice, the petroleum ether extract of the leaves of *Mentha arvensis* L., at the doses 10 and 20 mg/mouse per day for 20, 40 and 60 days, when administered orally, showed a dose and duration dependent reduction in the number of offspring of the treated male mated with normal females. Negative fertility was observed in both dose regimens after 60 days of treatment. The body weight and libido of the treated ammals remain unaffected. However, a significant decrease in the weight of the testis, epididymis, cauda epididymal sperm count, motility, viability and normal morphology of the spermatozoa was observed. The levels of serum protein, bilirubin, GOT, GPT and acid phosphatase, blood urea and haematological indices were unaltered throughout the course of investigation. All the altered parameters were reversible following withdrawal of treatment. The results suggest that the petroleum ether extract of the leaves of *M. arvensis* possess reversible antifertility property without adverse toxicity in male mice (Sharma and Jocob, 2001).

2.2 Phytochemical Prospection, Toxicity and Antimicrobial Activity of *Mentha arvensis* (Labiatae) from Northeast of Brazil

the ethanol extract of *Mentha arvensis* leaves, in order to evaluate its chemical composition, investigate it's in vitro antimicrobial potential against strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexineri*, *Klebsiella pneumonia* and *Staphylococcus aureus*, and find its toxicity toward Artemia salina and. Phytochemical analysis revealed the presence of catechic tannins, flavones, flavonols, xantones, flavonols, flavonones and steroids. The antibacterial activity is more significant against Sthaphylococcus aureus. In toxicity tests on *Artemia salina*, the ethanol extract from *Mentha arvensis* leaves showed LC50 value of 100 µg/mL. These results may justify the popular use of this species as it has antimicrobial activity and demonstrate its significant toxic effect on brine shrimp. However, in order to evaluate possible clinical application in therapy of infectious diseases, further studies about the safety and toxicity of isolated compounds are needed (Londonkar and Poddar, 2009).

2.3 Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals

Various crude extracts of petroleum ether, chloroform, or aqueous at a dose of 2 g/kg po did not produce any signs or symptoms of toxicity in treated animals. In the pyloric ligation model oral administration of different extracts such as petroleum ether, chloroform and aqueous at 375 mg/kg *po*, standard drug ranitidine 60 mg/kg *po* and control group 1% Tween 80, 5 mL/kg *po* to separate groups of Wister rats of either sex (*n* = 6) was performed. Total acidity, ulcer number, scoring, incidence, area, and ulcer index were assessed. There was a decrease in gastric secretion and ulcer index among the treated groups i.e. petroleum ether (53.4%), chloroform (59.2%), aqueous (67.0%) and in standard drug (68.7%) when compared to the negative control. It was found that pre-treatment with various extracts of *Mentha arvensis* Linn in three rat/mice ulcer models ie ibuprofen plus pyloric ligation, 0.6 mol/L HCl and 90% ethanol produced significant action against acid secretion (49.3 ± 0.49 *vs* 12.0 ± 0.57, *P*< 0.001). Pre-treatment with various extracts of *Mentha arvensis*Linn showed highly -significant activity against gastric ulcers (37.1 ± 0.87 *vs* 12.0 ± 0.57, *P*< 0.001). So it can be said thatVarious extracts of *Mentha arvensis* Linn. 375 mg/kg body weight clearly shows a protective effect against acid secretion and gastric ulcers in ibuprofen plus pyloric ligation, 0.6 mol/L HCl induced and 90% ethanol-induced ulcer models (Nascimento et al., 2009).

2.4 Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L

The inter-nodal and leaves segments of *M. arvensis*were cut into 0.5-0.7 cm in length and cultured on Murashige and Skoog solid medium supplemented with 3% sucrose, gelled with 0.7% agar and different concentration of 2, 4-Dichlorophenoxyacetie acid (2,4-D) either alone or in combinations. The preliminary phytochemical screening was performed by Brindha *et al* method. Antibacterial efficacy was performed by disc diffusion method and incubated for 24 h at 37 °C. Maximum percentage of callus formation (inter-nodal segments 84.3 ± 0.78; leaves segments 93.8 ± 1.27) was obtained on Murashige and Skoog's basal medium supplemented with 3% sucrose and 1.5 mg/L of 2, 4-D. The ethanol extracts of leaves derived calli showed the maximum bio-efficacy than other solvents. The leaves and stem derived calli extracts on *Proteus* sp. showed that the plants

can be used in the treatment of urinary tract infection associated with *Proteus* sp. Through the bacterial efficacy studies, it is confirmed that the *in vitro* raised calli tissue was more effective compared to *in vivo* tissue. The bio-efficacy study confirmed that the calli mediated tissues showed the maximum zone of inhibition. The present study paved a protocol to establish high potential cell lines by *in vitro* culture (Johnson et al., 2011).

2.5 Comparative study of *Mentha arvensis* Linn whole plant extracts for antioxidant and antidepressant activity

Mental disorders contribute significantly to the global burden of diseases. Projections towards the year 2020 show that the neuropsychiatric illness will increase their share from about 10.5% of the total burden of disease in 1990 to 15% in 2020 . *Mentha arvensis* is an indigenous plant of the Lamiaceae family commonly known as '*Pudina*' and '*Mint*' in English. It is reported to exhibit central nervous system (CNS) modulating effects . Hence, the present study was carried out to investigate aqueous and methanol extracts of *Mentha arvensis* Linn extracts for antioxidant and antidepressant activity. Aqueous and methanol extracts of *Mentha arvensis* were preliminary studied for *in vitro* antioxidant activity using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Nitric oxide and Hydroxyl radical scavenging activity methods. Then the extracts were investigated for antidepressant activity by Tail suspension and Forced swim test at dose level of 125, 250 and 500mg/kg in Swiss albino mice. Fluoxetin was used as a positive control at the dose of 10mg/kg. Methanol extract showed significant antioxidant and antidepressant activity as compared to aqueous extract. However, there is need for further studies to evaluate its mode of action (Tupe et al., 2010).

2.6 Antimicrobial activity of *Mentha arvensis* against clinical isolates of human cariogenic pathogens an in-vitro study.

Patients with chronic dental infection are usually treated with antibiotics. However, the value of antibiotics was decreasing because increased resistance in bacteria. The objective of this study is to evaluate the efficacy of herbal crude extract of *Mentha arvensis* in human Cariogenic pathogens. In this study we obtained crud extract of *Mentha arvensis* in different solvent 50% and 10% methanol, ethyl acetate, chloroform and was tested

against human Cariogenic pathogens *Streptococcus mutans, Streptococcus sangunis, Staphylococcus aurues, Lactobacillus casei* were isolated from patients having dental disease. The crude extracts activity were studied by disc diffusion and both dilution methods in different concentration. Studies were also undertaken to assess the phytochemical composition of the *Mentha arvensis* extract. 50% methanolic extract at 2.5mg/ml and 5mg/ml concentration shows slightly bigher zone of inhibition (ranging from 26 to 30 mm and 28 to 32 mm), and 10% methanolic 2.5mg/ml and 5mg/ml extract shows slightly small zone (ranging from 20 to 24 mm and 22 to 27 mm) and comparison with ethyl acetate and chloroform shows small zone at 5mg/ml ranging from 15 to 18 mm and 13 to 17 mm and in 2.5gm/ml ranging from 14 to 15mm and 09 to 16 mm or to be moderately sensitive. MIC results exhibit the profound and promising activity of *Mentha arvensis* on BHI 0.090 mg/ml. The secondary metabolites commonly present in the test leaves are Alkaloids, Tannins, Flavonols, Steroids, Xantones and glycosides, The GCMS analysis of revealed, the presence of Eucalyptol, Isomethone, Linalool, methnol, 4-Terpineol, OleicAcid, Tetradecanoic acid, 12-methyl-, methyl ester, Hexadecanoic acid, (Palmitic acid) methyl ester. These data suggest that extracts of *Mentha arvensis* contain significant amounts of phytochemicals with antioxidative properties which could serve antimicrobial property of the *Mentha arvensis* and it is exploited as a potential source for plant-based pharmaceutical products. These results could form a sound basis for further investigation in the potential discovery of new natural bioactive compound (Dwin, 2009)

2.7 Taxonomic studies on *Mentha arvensis* L. in Finland

The chromosome number of Finnish *Mentha arvensis* L. is 2n = 72. The material consists of 11 strains from different parts of the country and from different habitats, and includes the whole range of morphological variation in Finland. 2n = 72 agrees well with previous counts from the species in other countries. 2. With the aid of cultivation experiments on 70 strains the correlations between morphological (etc.) characters have been studied, as well as the persistence of the characters and, on the other hand, the effect of environment on them. 3. On the maps and in the tables are to be seen some vegetative and floral characters with their geographical distribution and relationships to habitat, especially to natural and man-made ones. 4. Within the species *Mentha arvensis* L. in Finland four infraspecific units have been separated, linked almost continuously with each other

through numerous intermediates. The characters, distribution, and ecology of these units have been described. However, these units have no taxonomic names or taxonomic status so far. Nevertheless, previous names have been mentioned when they are regarded as corresponding well with the units above. In my opinion it would be best to treat these four infraspecific units as varieties. 5. Some obvious weed clones have been found, with rather extensive distribution as a result of vegetative propagation by rhizomes transferred with garden trees and bushes and perennial plants, and some others are suspected. One (or a group) of these, in northern Finland, is clearly an old but nowadays neglected cultivated plant relic, being a northern vicariant for the earlier known nothomorphs of Mentha × gentilis L. and for M. × dalmatica Tausch. This northern *M. arvensis* has recently been included in the cultivation experiment (Jstor.org, 2016).

2.8 Anticancer activity of *Mentha arvensis*

The comparative study of antioxidant activity, total flavonoids and phenol content of these three plants species (Mentha arvensis, Elettaria cardamomum and Allium porrum) were studied. *Mentha arvensis* was found to contain more antioxidant,total flavonoids and phenol content when compared to others. Ethanolic extracts of *Mentha arvensis* was studied for the invitro cytotoxicity against Human liver cancer (Hep G2 cell line).*Mentha arvensis* treated Hep G2 cell lines with maximum cytotoxicity(46.3 µg $mL^{-}1$).The finding of the present investigation demonstrated that *Mentha arvensis* significantly suppresses growth and induces apoptosis in Hep G2 cell lines by MTT assay (Kumar Chandan, 2014).

2.9 Comparative study of qualitative phytochemical screening & antioxidant activity of *Mentha arvensis*, *Elettaria cardamonum*&*Allium porrum*.

In the current study, we carried out a comparative analysis of qualitative phytochemical screening and the antioxidant activities of 75% Ethanol, Acetone, Chloroform, Petroleum ether and aqueous extracts of the selected species (Mentha arvensis, Allium porrum and Elettaria cardamomum) was studied. Five solvents via; 75% Ethanol, Acetone, Chloroform, Petroleum ether and aqueous were used to obtain extracts from powdered plants parts. The extracts were subjected to qualitative Phytochemical screening using

standard procedures. Antioxidant activity of the five extracts using free radical scavenging assays like DPPH was determined. It was observed that presence of greater amount of phenolic compounds leads to a more powerful radical scavenging effect as was shown by ethanolic extract of the leaves and seeds when compared to the other extracts investigated by us during flavonoids and phenol test. *Mentha arvensis* leaves exhibited the maximum content of phenols and flavonoids compounds hence greatest antioxidant profile when compared to two other plants). (Tupe et al., 2010).

2.10 Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial Parts of *Mentha arvensis* Linn.

The present study deals with the macroscopical and microscopical studies of aerial parts of Mentha arvensis Linn. Microscopically, aerial parts showed glandular trichomes, helical to spiral xylem, palisade tissues with columnar cells, diacytic stomata. Powder microscopical examination showed the presence of glandular and uni to multi celled trichomes, helical to spiral xylem vessel, stomatal epidermal cells, abundant xylem vessels with pitted thickenings, abundant thin walled parenchymatius cells, epidermis with cuticle and collenchymatous cells, parenchymatous cells with reddish tannin contents. Physicochemical parameters and preliminary phytochemical studies of the powdered aerial parts were also carried out. Total ash was approximately sixteen and four times more than acid insoluble and water soluble ash, respectively. Water soluble extractive was slightly higher than ethanol soluble extractive. T.L.C. of petroleum-ether, chloroform and ethanol extract showed eight spots, nine spots and six spots, respectively. Phytochemically, it exhibited alkaloids, glycosides, steroids and sugars. These findings might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs (Jhade et al., 2011).

2.11 In vitro study on the antimicrobial effect of hydroalcoholic extracts from *Mentha arvensis* L. (Lamiaceae) against oral pathogens

In vitro tests could be a valuable tool for the evaluation of medicinal plants' antimicrobial activity. Mentha arvensis of the Lamiaceae family is one of the most frequently

traditional plants used in Brazil. Hydroalcoholic extracts of *M. arvensis* were analyzed for antimicrobial activity on Streptococcus mutans, Streptococcus sobrinus and Candida albicans. Three different assays (agar diffusion, broth macro- and microdilution methods) were used to evaluate antimicrobial activity.Although hydroalcoholic extracts of M. arvensis did not show any antibacterial effect, its antifungal activity against C. albicans was revealed. According to the micro-dilution broth assay, MIC of the hydroalcoholic extract from leaves of M. arvensis on Candida albicans strains ranged between 625 and 2500 μg mL-1. Results suggest that M. arvensis hydroalcoholic extract may be considered a potentially antifungal agent against C. albicans, and a possible item for human antibiotic therapy. However, further biological tests on the plant's efficacy and side-effects are necessary before its use on humans . (Lund et al., 2012).

2.12 To Study the Anticancer and Apoptotic Activity of *Mentha arvensis*. Linn in ROS Induced Buccal cells.

The anticancer activity tested has shown notable activity against the cell lines. The present study confirms that the plant will be a potential source of medicine in future and extensive study is required for the discovery of new drugs on the active biological molecules. The methanolic leaf extract *Mentha arvensis* has shown significant cytoprotective activity too. The cell treated with H2O2 has a reduced viability than the methonolic leaf extract treated cells. In H2O2 treated cells the cytotoxicity was more than 70% i.e. only 34.76 % is viable and the cells treated with the methanolic leaf extract of *M. arvensis* has shown viability up to 54.23 %. It can be observed that addition of hydrogen peroxide produced approximately 79.3 % increase in DNA fragmentation which can be attributed to the DNA lesions formed by breaking phosphodiester bond in intact chromosomes by OH radicals. The plant extracts produced 63.7% decrease in DNA fragmentation indices suggesting potent cytoprotective effect against deleterious effects of hydrogen peroxide (Pattiyappan, 2014).

2.13 Antioxidant potential of methanol Root extract of *Mentha arvensis* L. from Kashmir Region

The study was carried out to evaluate the phytochemical constituents and antioxidant potential of methanolic root extract of *Mentha arvensis* L. from Kashmir region. The

antioxidant activity of methanol extract of *Mentha arvensis* L. was evaluated by using 1, 1-diphenyl, 2-picrylhydrazyl (DPPH) scavenging, reducing power, metal chelating, nitrous oxide scavenging and hydrogen peroxide scavenging assays. The total phenolic content and total flavonoid content was found to be 9.12 and 32.14 mg/g respectively. The percentage inhibition values of DPPH scavenging for methanol extract were found to be 35.83%. Thepercentage inhibition values of Nitrous oxide scavenging for extract were found to be 39.11%, the percentage inhibition values ofH2O2scavenging for extract were found to be 21.72%, the percentage inhibition values of metal chelating activity of methanol extract at the concentration of 500 µg/mlwas found to be 79.31% and that for standard ascorbic acid was found to be 96% at same concentration and the reducing power of extractwas found to be 0.75 at concentration of 0.25mg/ml. The results indicate that the methanolic root extract of *Mentha arvensis* L. has good antioxidant potential and it can be regarded as promising candidates for natural plant sources of antioxidants with high (Patilap, 2012)

2.14 Antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of *Mentha arvensis* L.

In vitro DPPH radical scavenging assay was used to evaluate the antioxidant activity of the plant extract. *In vivo* analgesic activity was carried out by acetic acid-induced writhing test in Swiss albino mice. All studies in mice were undertaken at the doses of 250 and 500 mg/kg body weight. Antibacterial activity was studied by disk diffusion assay against some Gram-positive and Gram-negative bacterial strains. Brine shrimp lethality assay was used to investigate cytotoxicity effects of the plant extract. The extract showed free radical scavenging activity in the DPPH assay (IC_{50}~41 µg/mL) compared to the standard antioxidant ascorbic acid (IC_{50}~19 µg/mL). The extract also produced prominent antimicrobial activity against *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydii*, *Streptococcus pyogenes* and *Streptococcus aureus* compared to standard drug kanamycin at the dose of 30 µg/disc. The extract exhibited lethality against the brine shrimp nauplii with the LC_{50} values of 40 µg/mL, and also 90% mortality (LC_{90}) value was found to be 160 µg/mL. In analgesic test, the extract demonstrated statistically significant (P<0.01) analgesic effect in acetic acid induced writhing in white albino mice at both dose levels. These results suggest that the ethanolic extract of *Mentha arvensis* L.

has potential antioxidant, antibacterial, cytotoxic and analgesic activities that support the ethnopharmacological uses of this plant . (Biswas, Saha and Ali, 2014).

2.15 Fragrance Chemicals in the Essential Oil of *Mentha arvensis* Reduce Levels of Mental Stress

The aim of this work was to determine the chemical composition of essential oil from aerial partsof *Mentha arvensis* L. f. *piperascens* (MAO) and to evaluate the effect of its fragrant chemicals on electro-encephalographic (EEG) activity of human brain. The MAO was obtained by supercritical CO2 extraction. The maximum yield was 2.38% at conditions of 70^{o}C and 200 bar. There were 32 volatile chemicals with 6 alcohols (67.11%), 13 hydrocarbons (17.05%), 9 esters (11.50%), 2 ketones (7.16%), 1 oxide (2.77%), and 1 aldehyde (0.56%). The major components were (*Z*,*Z*,*Z*)-9,12,15-octadecatrien-1-ol (50.06%), 2-hydroxy-4-methoxyacetophenone (7.50%), and 3,4-dihydro-8-hydroxy-3-methyl-1H-2-ben-zopyran-1-one (6.60%). Results of the EEG study showed that inhalation of MAO significantly changed the EEG power spectrum values of relative gamma, relative fast alpha, and spectral edge frequency 90%. During the inhalation of MAO, the value of relative fast alpha was significantly increased (p<0.05). On the other hand, the values of gamma and the spectral edge frequency 90% were significantly decreased (p<0.05). The present study suggests that fragrant chemicals of essential oil of *M.arvensis* reduce the level of mental stress and that they could be used in the treatment of psychophy-siological disorders (Cho et al., 2013).

2.16 *In vitro thrombolytic & cytotoxic evaluation of Mentha arvensis* L., *Mentha spicata* L. and *Mentha viridis* L.

Atherothrombotic diseases or coronary artery thrombosis are common disorders which are treated bystreptokinase (SK), urokinase (UK) or tissue plasminogen activators (t-PA). Because of the high risk of bleeding, severe anaphylactic shock, intracranial hemorrhage and lacks of specificity of these drugs, they are restricted to those patients whohave undergone surgery or those with a history of gastrointestinal bleeding or hypertension. Therefore, plant based drugs are used because they are cheap, safe, low side effects and effective against many diseases.The study was carried out to check the clot lysis effect and cytotoxic effects ofmenthaarvensis L., Menthaspicata L. And menthaviridisl.using

streptokinase as a positive control and water as a negative control. In this experiment, the M. Arvensis, M.spicata and M. Viridisshowed 32.56%, 30.89%, 30.29% clot lysis activity in case of methanol extract, 32.04%, 30.37%, 30.02% clot lysis activity in case of ethanol extract, 31.87%, 29.77%, 29.05%clot lysis activity in case of chloroform extract and 30.29%, 28.45%, 27.55% clot lysis activity in case of acetone extract respectively. In brine shrimp cytotoxic assay the methanol extractsm. Arvensis, M. Spicata and M. Viridis showed LC_{50} values of 2.088, 1.964 and 1.812 µg/ml, respectively, which was referred to Vincristine sulfate (LC_{50}1.160).From this study, it can be said that the M. Arvensis, M.spicata and M.viridis has clot lysis activity and low cytotoxic activity. So, these plantscould be incorporated as a thrombolytic agent with In vivo effects to improve the atherothrombotic patients (Shahik et al., 2014).

Chapter 3 : Methods & Materials

3.1 Collection and preparation of plant material

Plant sample of *Mentha arvensis* was collected from Dhaka in October, 2015. Then proper identification of plant sample was done by an expert taxonomist. The plant was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried plant was then ground in coarse powder using high capacity grinding machine.

3.2 Extraction of the plant material

About 650 gm of the powdered material was taken in separate clean, round bottomed flask (5 liters) and soaked in 3.5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 390°C with a Heidolph rotary evaporation.

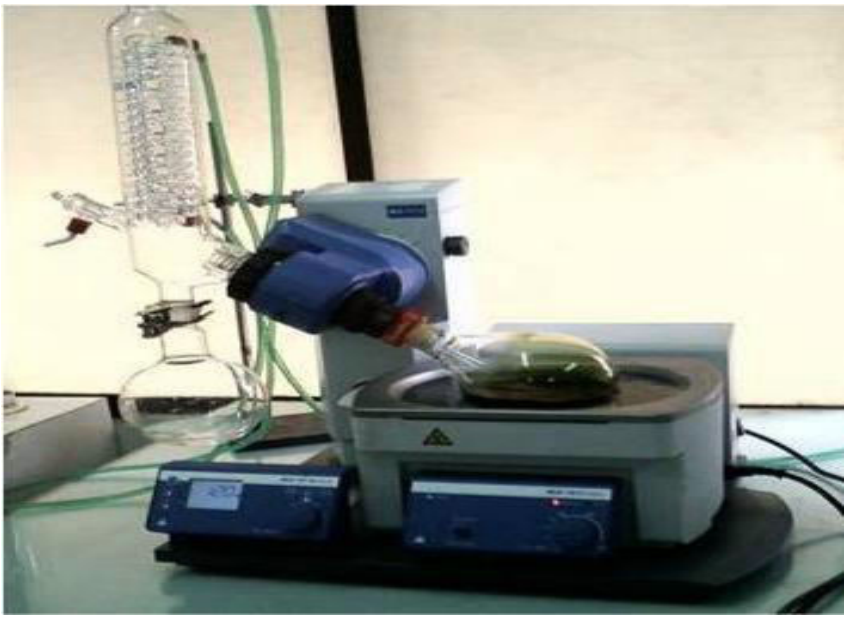


Figure 3.1: Drying of extract using rotary evaporator

The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 25.18 gm respectively.

3.3 Preparation of Mother Solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This is the mother solution.

3.4 Partition of Mother Solution

The mother solution was then partitioned off successively by three solvents of different polarity.

3.4.1 Partition with Pet-ether

The mother solution was taken in a separating funnel. 100 ml of the Pet-ether was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml X 3). The Pet-ether fraction was then air dried for solid residue.

3.4.2 Partition with Dichloromethane

To the mother solution left after partitioning with Pet-ether, 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with Dichloromethane (DCM). The process was repeated thrice (100 ml X 3). The DCM fraction was then air dried for solid residue.

3.4.3 Partition with Ethyl acetate

To the mother solution that left after washing with Pet-ether, and Dichloromethane, 16 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with ethyl acetate. The process was repeated thrice (100 ml X 3). The ethyl acetate fraction was then air dried for solid residue.

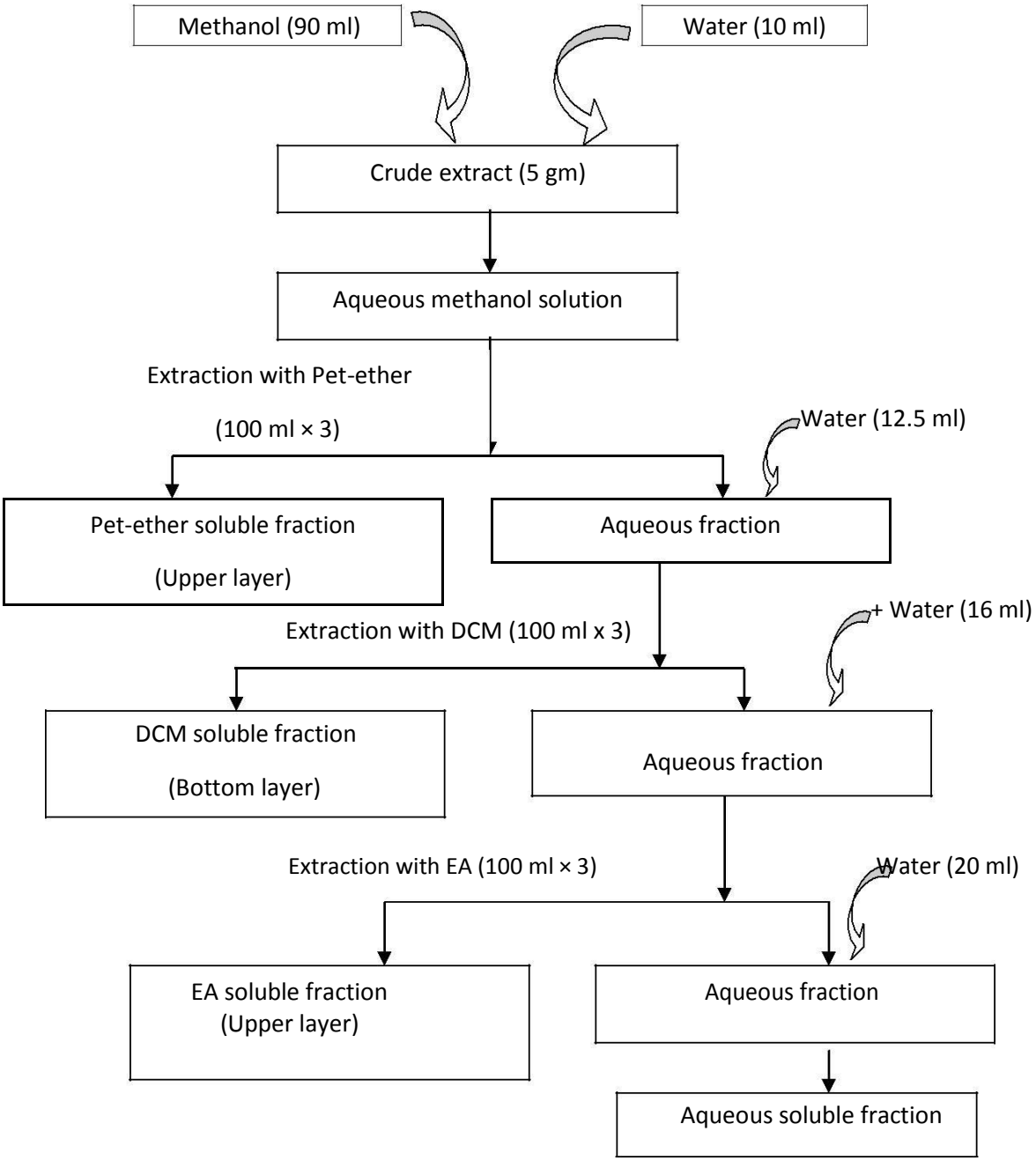


Figure 3.2: Schematic representation of the partitioning of Methanolic crude *Mentha arvensis*

3.4.4 Collection of Pet-ether Fraction

After partitioning the mother solution with the three different solvents the Pet-ether fraction was collected and air dried. This aqueous was further investigated for different pharmacological properties such as Antioxidant and Cytotoxic (Beckett AH and Stenlake JB, 1986).

3.5 Brine Shrimp Lethality Bioassay

3.5.1 Principle

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (*in-vivo*) lethality, a simple zoological organism, (Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus *Artemia* of aquatic crustaceans. *Artemia* is the only genus in the family Artemiidae (Olowa *et al.*,2013)

3.5.2 Apparatus & Reagents

Table 3.1: Apparatus and reagents for Brine shrimp lethality bioassay

Artemia salina leach (brine shrimp eggs)	Pipettes & Micropipette
Sea salt (NaCl)	Glass vials
Small tank with perforated dividing dam to hatch the shrimp	Magnifying glass
Lamp to attract shrimps	Test samples

3.5.3 Procedure

3.5.3.1 Preparation of Sea Water

To hatch the brine shrimp nauplii for the assay, sea water representing brine should be prepared at first. To prepare sea water 38 gm of pure NaCl was dissolved in distilled water and then the volume made up to 1000 ml by distilled water in a 1000 ml beaker for *Artemia salina* hatching. 1-2 drops of 1 N NaOH or 1 N HCl solution was added with a dropper for obtaining the pH 8.4 as sea water.

3.5.3.2 Hatching of Brine Shrimp

A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. Then a dry preserved egg of *Artemia salina* Leach was added in the artificial sea water. Oxygen was supplied through an air pump and a table lamp was placed near the beaker. The eggs of *Artemia salina* were hatched at room temperature (25-30ºC) for 18-24 hours. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. 10 living shrimps were then collected by a pipette and then added to each of the test tubes containing 5 ml of seawater. Those freshly hatched free-swimming nauplii were used for the bioassay (Niazi J. *et al*., 2009).

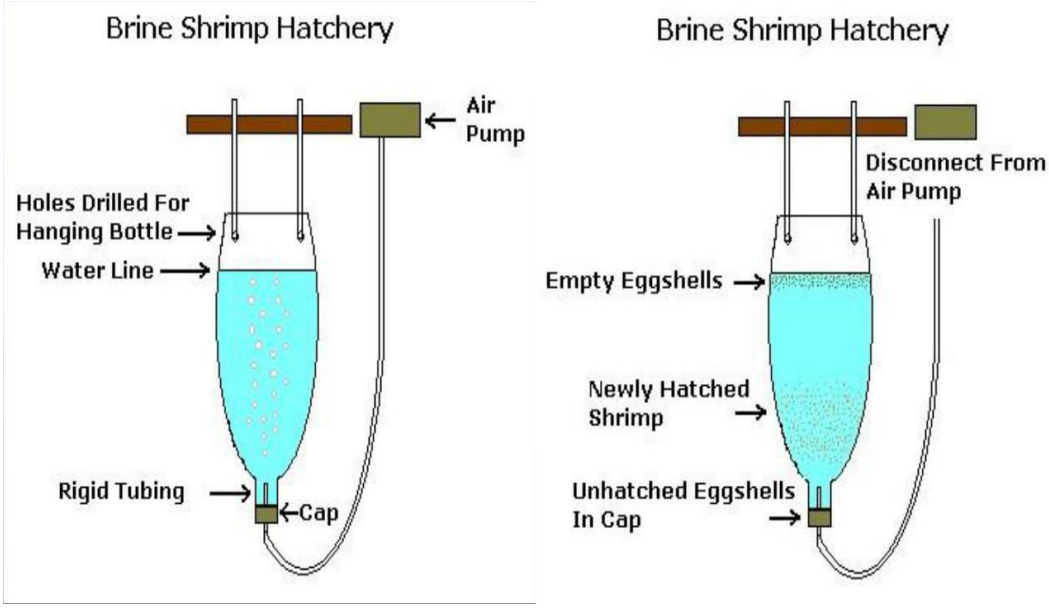


Figure 3.3: Brine shrimp Hatchery

3.5.3.3 Preparation of Test Solutions

Clean test tubes were taken. These test tubes were used for ten different concentrations (one test tube for each concentration) of test samples and ten test tubes were taken for standard drug tamoxifen for ten concentrations of it and another one test tube for control test.

3.5.3.4 Preparation of the Test Samples of Experimental Plant

All the test samples of 4 mg were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) in vials to get stock solutions. Then 100 µl of solution was taken in test tube each containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100 µl sample was added to test tube and fresh 100 µl DMSO was added to vial. Thus the concentrations of the obtained solution in each test tube were 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml for 10 dilutions.

3.5.3.5 Preparation of the Positive Control Group

In the present study tamoxifen is used as the positive control. Measured amount of the tamoxifen is dissolved in DMSO to get an initial concentration of 2000 µg/ml. From that stock solution serial dilutions are made using DMSO to get 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml. Then ten living brine shrimp nauplii in 5 ml simulated seawater are added to the positive control solutions in the pre-marked test-tubes to get the positive control groups.

3.5.3.6 Preparation of the Negative Control Group

100 µl of DMSO was added to the pre-marked test tube containing 5 ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds (Goldstein *et al.*, 1974).

3.5.3.7 Counting of Nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration (Sleet RB and Brendel K, 1983).

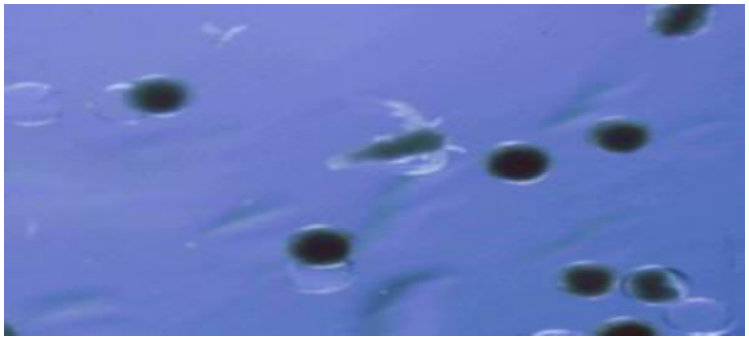


Figure 3.4: Counting of nauplii

3.6 Antioxidant Activity

3.6.1 Total Phenolic Content

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, it has been reported that there is an inverse relationship between the antioxidative status occurrences of human diseases. In addition, antioxidant compounds which are responsible For Such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders. Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the compounds, of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid per oxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidative effects have rarely been carried out. The purpose of this study was to evaluate extractives of *Opuntia elatior* as new potential sources of natural antioxidants and phenolic compounds. This

study also demonstrates a possible relationship between phenolic content and antioxidant activity.

3.6.1.1 Principle

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–

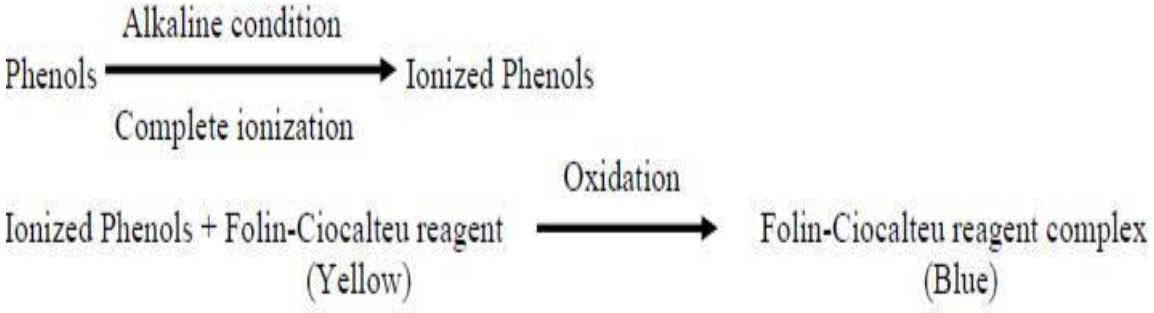
Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. In the alkaline condition phenols ionize completely.

Table 3.2: Composition of 100 mg Folin-Ciocalteu Reagent

i.	Water	57.5 ml
ii.	Lithium Sulfate	15.0 mg
iii.	Sodium Tungstate Dihydrate	10.0 mg
iv.	Hydrochloric Acid (25%)	10.0 mg
v.	Phosphoric Acid 85% solution in water	5.0 mg
vi.	Molybdic Acid Sodium Dihydrate	2.5 mg

When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates - molybdates. Sequences of reversible one-or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$.

The intensity of the color change is measured in a spectrophotometer at 765 nm. The absorbance value will reflect the total phenolic content of the compound (Singleton *et al.*, 1999).



3.6.1.2 Apparatus & Reagents

Table 3.3: Apparatus and reagents used for total phenolic content

Folin-Ciocalteu reagent (10 fold diluted)	UV-spectrophotometer
Ascorbic acid	Beaker (100 & 200 ml)
Na_2CO_3 solution (7.5%)	Test tube
Methanol	Micropipette (50-200 µl)
Distilled water	Cuvette

3.6.1.3 Procedure

Standard curve preparation: Ascorbic acid was used here as standard. Different ascorbic acid solutions were prepared having a concentration ranging from 120 µg/ml to 80 µg/ml. 5 ml of FCR (diluted 10 times with water) and 4 ml of Na_2CO_3 (7.5% w/v) solution was added to ascorbic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 765 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

Sample preparation: 2 mg of the *Mentha arvensis* pet-ether fraction was taken and dissolved in 1ml methanol to get a sample concentration of 2 mg/ml.

Determination of total phenol content

- 1.0 ml plant extract of different concentrations (120 µg/ml, 110 µg/ml, 100 µg/ml, 90 µg/ml and 80 µg/ml) was taken in test tubes.
- 5 ml of Folin–ciocalteu (Diluted 10 fold) reagent solution was added into the test tube.
- 4 ml of Sodium carbonate solution was added into the test tube.
- The test tubes containing the samples were incubated for 1 hour at the room temperature to complete the reaction.
- Absorbance of solution was measured at 765 nm using a spectrophotometer against blank.
- A typical blank solution containing methanol was taken.

3.6.2 Total Flavonoid Content

3.6.2.1 Principle

Aluminium chloride ($AlCl_3$) colorimetric method is incorporated to determine the total flavonoid contents of the crude plant extract. The basic principle of the assay method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols of the crude extract. In addition aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A or B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorbance maximum at 510 nm. Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 510 nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard (Chang C *et al.*, 2002).

Flavonoid (Extract) + $AlCl_3$ (reagent) = Formation of flavonoid-aluminium complex

(λ_{max} = 510 nm)

3.6.2.2 Apparatus & Reagents

Table 3.4: Apparatus and reagents used for total flavonoid content

Aluminium chloride	Spatula
Methanol	Analytical balance
Ascorbic acid	Pipette and pumper
Sodium hydroxide	Aqueous fraction
Sodium nitrite	Test tubes and beaker

3.6.2.3 Procedure

Preparation of 10% Aluminium Chloride ($Alcl_3$) Solution: 10 mg of $AlCl_3$was taken into a100 ml of a volumetric flask and the volume was adjusted by distilled water.

Preparation of 4% NaOH Solution: 4 mg of NaOH was taken into a 100 ml volumetric flaskand the volume was adjusted by distilled water.

Preparation of 5% (W/V) $NaNO_2$ Solution: 5 mg of $NaNO_2$was taken into a 100 ml of avolumetric flask and the volume was adjusted by distilled water.

Preparation of Standard Solution: The stock solution was prepared by taking 0.025 gm ofascorbic acid and dissolved into 5 ml of ethanol. Concentration of this solution was 5000 µg/ml of ascorbic acid. The experimental concentrations were prepared from this stock solution.

Table 3.5: Preparation of standard solution

Concentration (µg/ml)	Solution taken from stock solution (µl)	Volume adjusted by methanol (ml)	Final volume (ml)
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

Preparation of Extract Solution: 5 ml of each plant extracts were taken and dissolved into 5 ml of methanol. The concentration of the solution was 1 mg/ml of plant extracts. Then the following steps were carried out. 1.5 ml extract was taken in a test tube and then 6 ml of distilled water was added. Then 5% of $NaNO_2$was added and incubated for 6 minutes. 10% $AlCl_3$ was added and incubated for 6 minutes. 4% NaOH and 0.6 ml distilled water was added. Then it was incubated for 15 minutes. For blank solution 1.5 ml methanol was taken and same procedure was repeated.

Then the absorbance of the solution was measured at 510 nm using a spectrophotometer against blank

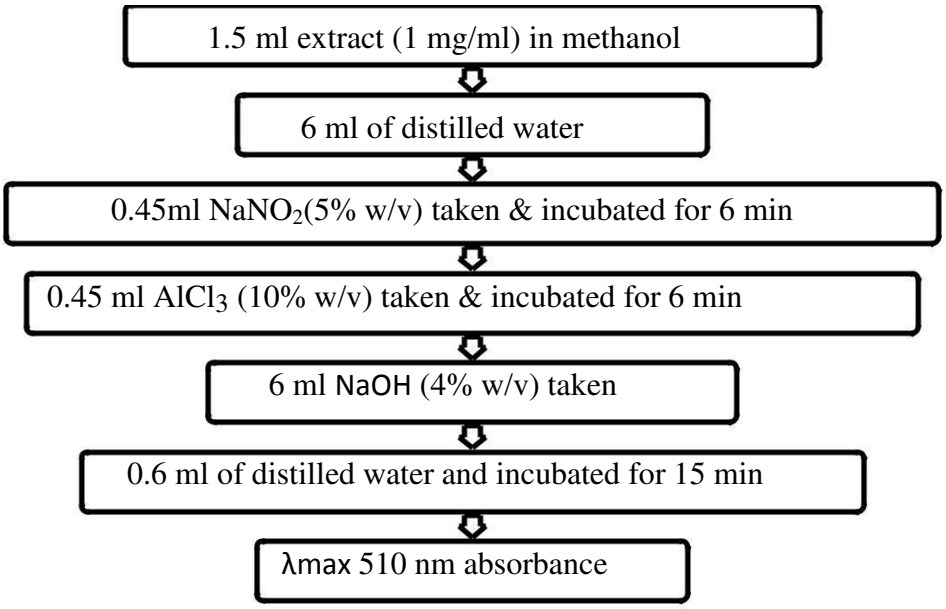


Figure 3.5: Schematic diagram of preparation of extract solution

Preparation of blank solution:

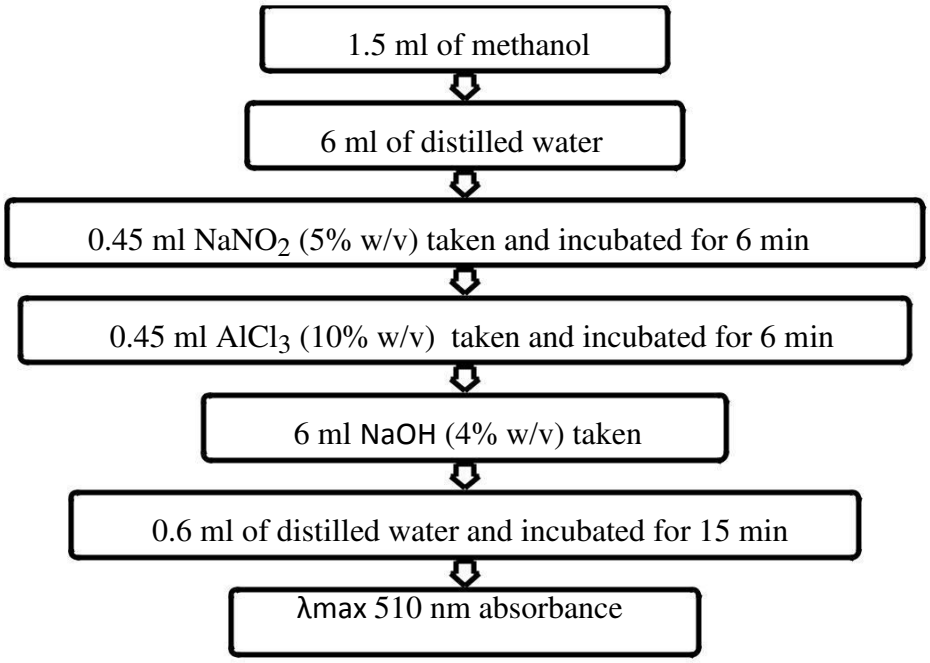


Figure 3.6: Schematic diagram of preparation of blank solution (Zhishen J*et al*., 1999).

3.6.3 Reducing power assay

3.6.3.1 Principle

The reducing power of petroleum ether extract of *Mentha arvensis* was determined by the method of Oyaizu. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + ferrous chloride

3.6.3.2 Apparatus and reagents

Table 3.6: Apparatus and reagents used for Reducing power assay

Spatula	Potassium ferricyanide
Analytical balance	Methanol
Pipette and pumper	Ascorbic acid
Aqueous fraction	Trichloro acetic acid
Test tubes	Phosphate buffer
Beaker	Ferric chloride

3.6.3.3 Procedure

Phosphate buffer (0.2 M, pH 6.6) preparation

Dibasic sodium phosphate (18.75 ml of 0.2M) is mixed with 31.25 ml monobasic sodium phosphate and diluted to 100 ml with water.

Potassium ferricyanide (1% w/v) preparation

1 mg of potassium ferricyanide (K_3 [Fe (CN) $_6$]) was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

Trichloro acetic acid (10%) preparation

10 mg of trichloro acetic acid (CCl_3COOH) was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

Ferric chloride (0.1%) preparation

0.1 mg of ferric chloride ($FeCl_3$]) was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

Standard solution preparation

The stock solution was prepared by taking 0.025 gm of ascorbic acid and dissolved into 5 ml of methanol. The concentration of this solution was 5000 µg/ml of ascorbic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Table 3.7: Different concentrations of ascorbic acid solution preparation

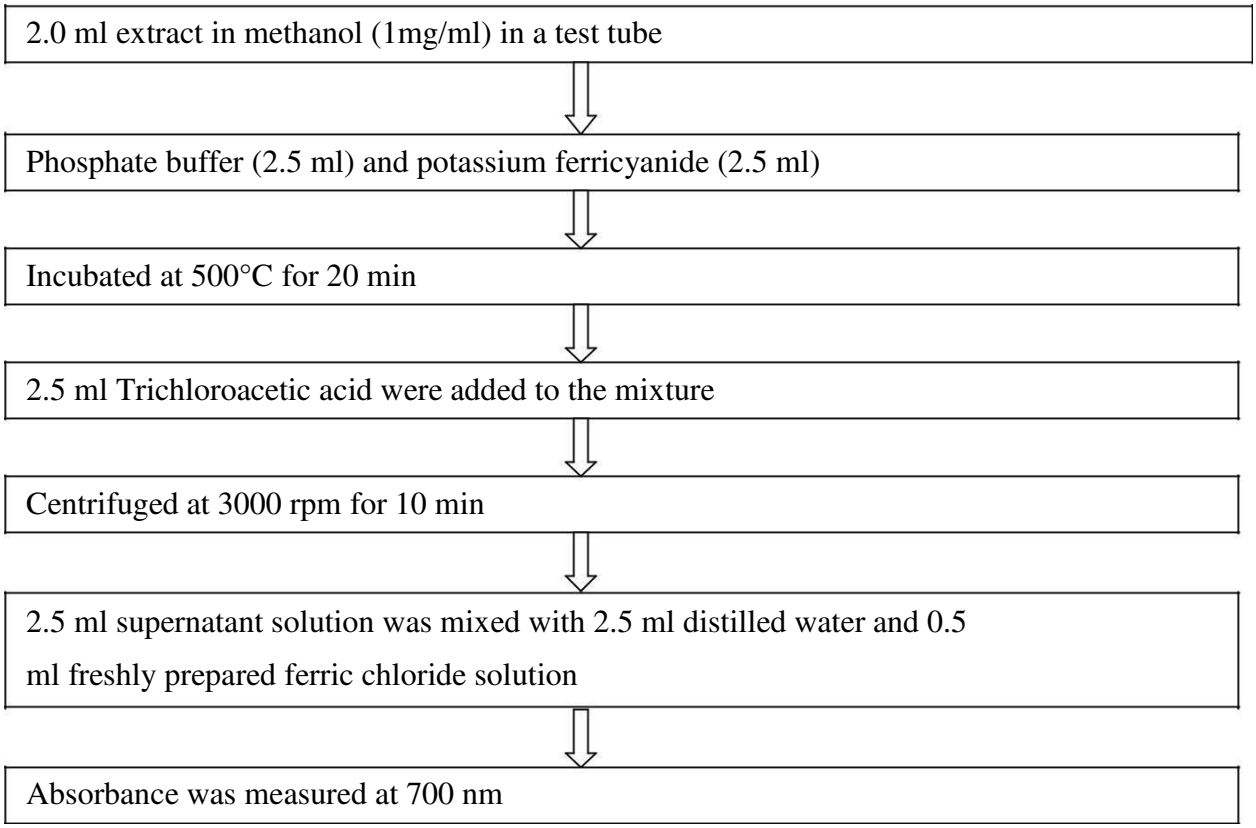
Concentration (µg/ml)	**Solution taken from stock solution (µl)**	**Volume adjusted by methanol (ml)**	**Final volume (ml)**
250	250	4.75	5
200	200	4.80	5
150	150	4.85	5
100	100	4.90	5
50	50	4.95	5

Extract solution preparation

5 mg of plant extract was taken and dissolved into 5 ml of methanol. The concentration of the solution was 1 mg/ml of plant extract.

Determination of reducing power

2.0 ml plant extract solution and ascorbic acid in different concentrations were taken in test tubes and mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 500°C for 20 min. 2.5 ml Trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. 2.5 ml upper layer (supernatant solution) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract.



$$\%\ \text{increase in Reducing Power} = (\frac{A_{test}}{A_{blank}} - 1) \times 100\%$$

Where A_{test} is absorbance of test solution; A_{blank} is absorbance of blank. Increased absorbance of the reaction mixture indicates increase in reducing power (Oyaizu M, 1986).

Chapter 4 : Results & Discussion

4.1 Result of Brine Shrimp Lethality Bio-Assay

The pet-ether fraction of the *Mentha arvensis* extract was subjected to brine shrimp lethality bioassay. After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors counted. The effectiveness of the concentration and % mortality relationship of plant product was expressed as a Median Lethal Concentration (LC_{50}) value. LC_{50} represents the concentration of the standard and aqueous extract that produces death in half of the test subjects after a certain period.

The percentage mortality at each concentration was determined using the following formula:

$$\% \text{ Mortality} = \frac{\text{Number of dead nauplii} \times 100}{\text{Total number of nauplii}}$$

The LC_{50} of the test samples was obtained by a plot of percentage of the shrimps died (% Mortality) against the logarithm of the sample concentration (Log C) and the best-fit line was obtained from the curve data by means of regression analysis.

4.1.1 Preparation of Curve for Standard

Here, Tamoxifen was used as reference standard.

Table 4.1: Results of the bioassay of Tamoxifen (standard)

Test tube no.	Concentration (C) (µg/ml)	LogC	Number of Naupliialive	Number of Naupliidead	% Mortality	LC_{50} (µg/ml)
1	400	2.602	0	10	100	
2	200	2.301	1	9	90	
3	100	2.000	2	8	80	
4	50	1.699	3	7	70	
5	25	1.398	4	6	60	13.38
6	12.5	1.097	5	5	50	
7	6.25	0.796	6	4	40	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	.078125	-0.107	9	1	10	

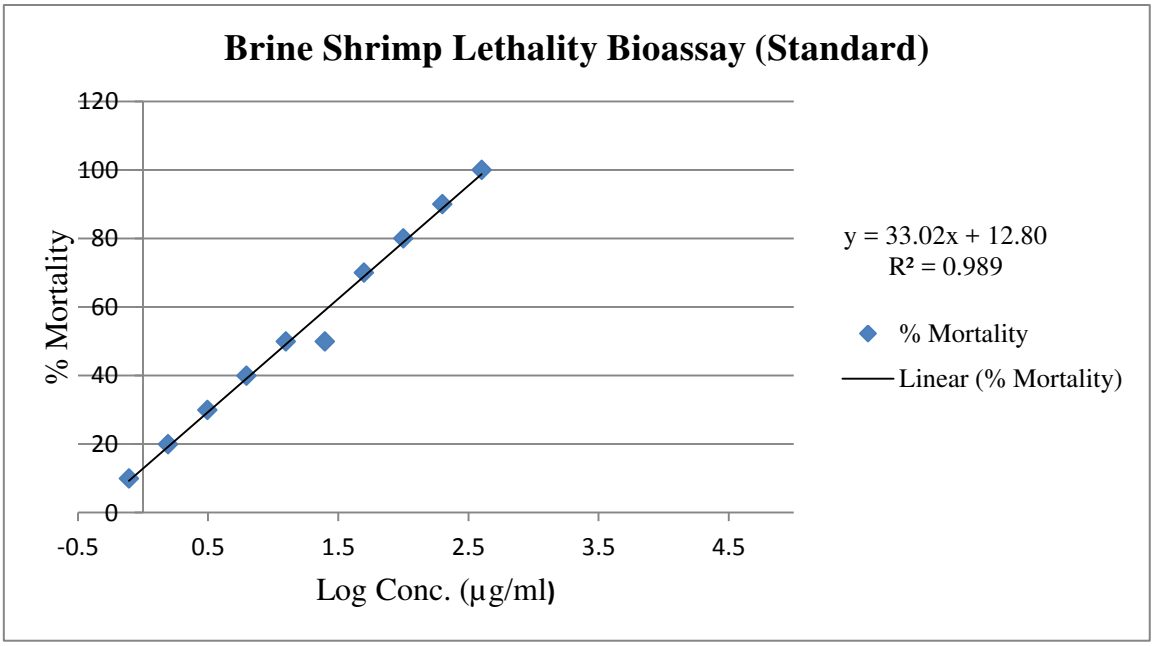


Figure 4.1: % mortality and predicted regression line of Tamoxifen (standard)

4.1.2 Preparation of Pet-ether Fraction Curve

Table 4.2: Results of the bioassay of pet-ether fraction (extract)

Test tube no.	Concentration (C) (µg/ml)	LogC	Number of nauplii alive	Number of naupliidead	% Mortality	LC_{50} (µg/ml)
1	400	2.602	0	10	100	
2	200	2.301	0	10	100	
3	100	2.000	1	9	90	
4	50	1.699	3	7	70	
5	25	1.398	4	6	60	
6	12.5	1.097	6	4	40	17.79
7	6.25	0.796	6	4	40	
8	3.125	0.495	7	3	30	
9	1.5625	0.194	8	2	20	
10	.078125	-0.107	8	2	20	

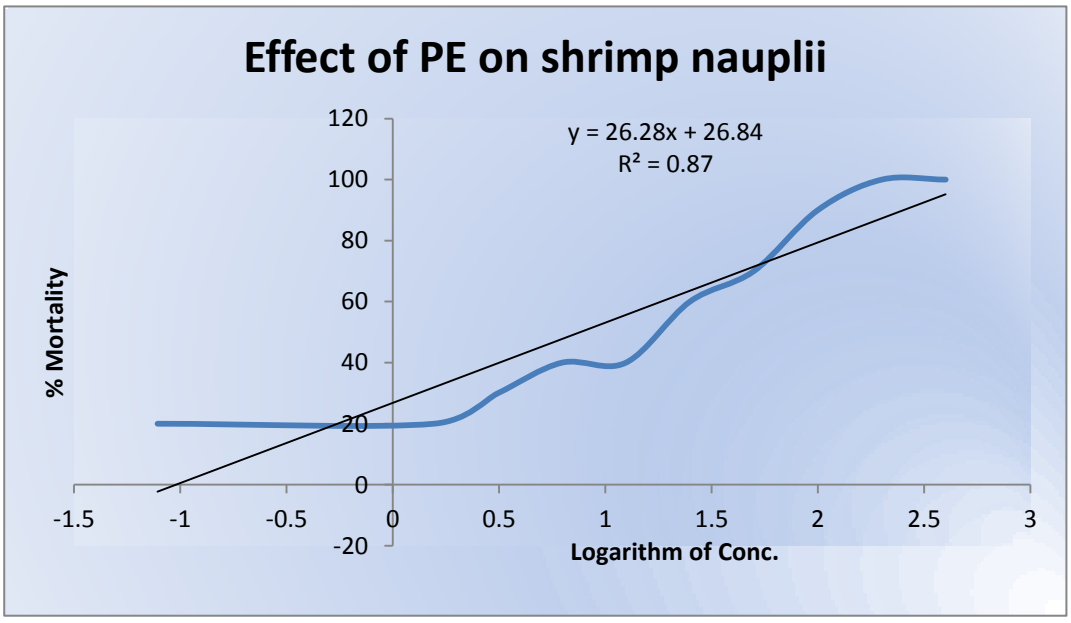


Figure 4.2: % mortality and predicted regression line of petroleum ether fraction (extract).

4.1.3Discussion

In Brine Shrimp Lethality bioassay, varying degree of lethality was observed with exposure to different concentrations of the test samples. The degree of lethality was found to be directly proportional to the concentration ranging from the lowest concentration to the highest concentration in both standard and pet-ether fraction samples. Mortality increased gradually with an increase in concentration of the test samples. Maximum mortalities took place at the highest concentration of 400µg/ml, whereas the least mortalities at lowest concentration 0.78125µg/ml as shown in Table 4.1 and Table 4.2.

Table 4.3: Cytotoxic activity of Tamoxifen and pet-ether fraction of *Mentha arvensis leaves*

Sample	Linear regression equation	R^2 value	LC_{50} (µg/ml, 24hr)
Standard (Tamoxifen)	y = 33.021x + 12.806	0.989	13.38
Extract (Pet-ether fraction)	y = 26.28x + 26.84	0.87	17.79

In this investigation, standard and pet-ether fraction exhibited cytotoxic activities with the LC_{50} values 13.38µg/ml and 17.64µg/ml respectively as shown in Table 4.3. For pet-ether fraction R^2 value is less than the standard which indicates that the extract has less potent activity than standard against brine shrimp nauplii.

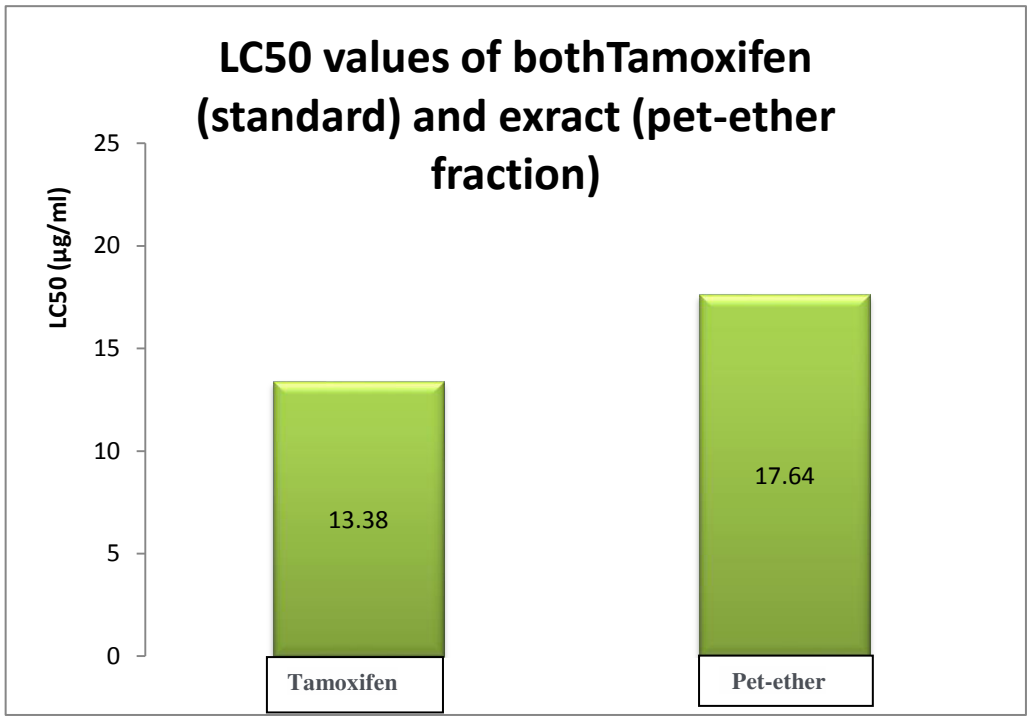


Figure 4.3: Comparison between LC_{50} values of standard and extract

From the above figure it can be concluded that for pet-ether fraction the lethal concentration required to kill 50% of the sample population is higher than the standard. So the extract is less potent than Tamoxifen (Standard) at lower concentration.

4.2 Result of Antioxidant Tests

Antioxidant tests are classified by various methods. Samples were subjected to various standard methods to determine various scavenging capacity and amount that is equivalent to the standard like ascorbic acids. Antioxidant property of the aqueous fraction of *Mentha arvensis* extract was determined by following methods:

- Determination of total phenolic content.
- Determination of total flavonoids content.
- Determination of total reducing power content.

4.2.1 Result of Total Phenolic Content

The pet-ether extract of *Mentha arvessis* was subjected to determine total phenolic content. Ascorbic acid was used as reference standard (Singleton *et al.*, 1999).

4.2.1.1 Preparation of Standard Curve

Table 4.4: Total Phenolic content of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 765 nm)**	**Regression line**	**R^2 value**
80	2.406	y = 0.019 x + 0.824	0.937
90	2.473		
100	2.767		
110	3.057		
120	3.080		

A linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.4. This linear curve was considered as a standard curve.

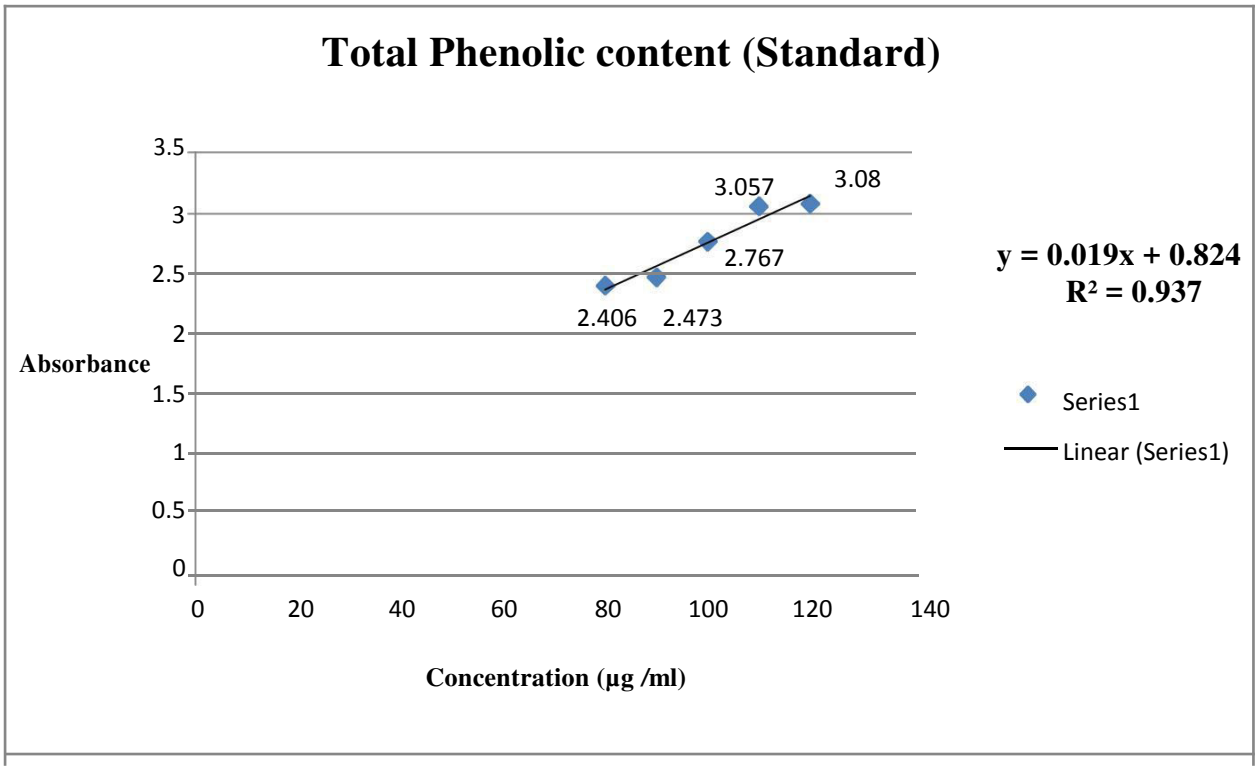


Figure 4.4: Graphical representation of Phenolic content of ascorbic acid

4.2.1.2 Total Phenolic content present in pet-ether extract of *Mentha arvensis*

Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of ascorbic acid equivalents (AAE), the total phenolic content present in the extract is calculated and given in the table below.

Table 4.5: Total Phenolic content in pet-ether fraction of *Mentha arvensis*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 765 nm)**	**Total Phenolic (X) value (mg of AAE/gm of dried extract)**
Aqueous fraction of *Mentha arvensis*	2	1.292	12.315

4.2.1.3 Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in phenolic content. Absorbance of the aqueous fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 12.315 mg of AAE/gm of dried extract of phenol content was found in the aqueous fraction of *Mentha arvensis*

4.2.2 Result of Total Flavonoid Content

The pet-ether fractions of *Mentha arvensis* were subjected to determine total flavonoid content. Ascorbic acid was used as reference standard (Chang C *et al.*, 2002).

4.2.2.1 Preparation of Standard Curve

Table 4.6: Total Flavonoid content of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 510 nm)**	**Regression line**	**R^2 value**
50	0.05	y = 0.001x-0.042	0.991
100	0.13		
150	0.19		
200	0.29		
250	0.39		

After absorbances were taken of different solution of ascorbic acid of concentrations ranging from 50µg/µl to 250µg/µl, a linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.5. This linear curve was considered as a standard curve.

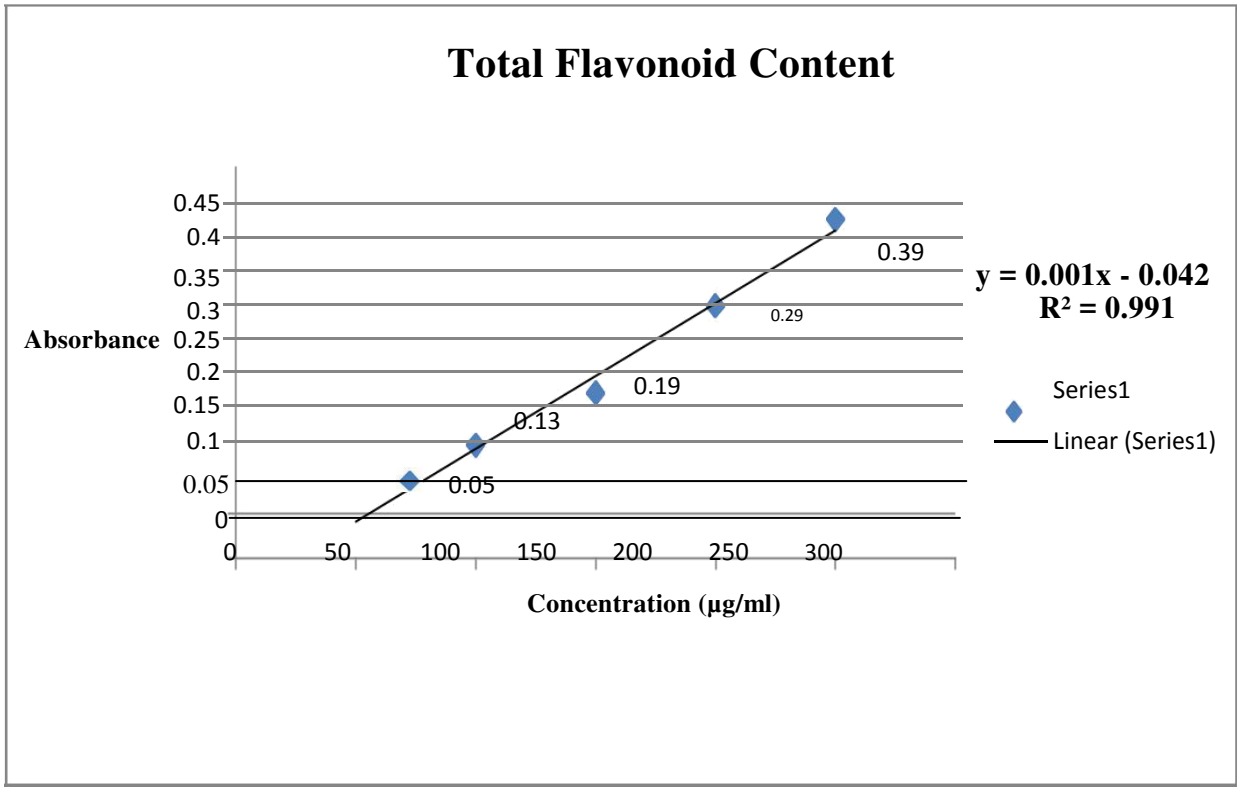


Figure 4.5: Graphical representation of Flavonoid content of ascorbic acid

4.2.2.2 Total Flavonoid Content Present in pet-ether fraction of *Mentha arvensis*

Based on the absorbance value of extract solution and using the regression line equation of the standard curve, the total flavonoid present in the extract is calculated and is given in Table 4.7.

Table 4.7: Total Flavonoid content in pet-ether fraction of *Mentha arvensis*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 510 nm)**	**Total Flavonoid (X) value (mg of AAE/gm of dried extract)**
Aqueous fraction of *Mentha arvensis*	1	0.014	56

4.2.2.3 Discussion:

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in flavonoid content. Absorbance of the pet-ether fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 56 mg of AAE/gm of dried extract of flavonoid content was found in the pet-ether fraction of *Mentha arvensis*

4.2.3 Result of Total Reducing Power Assay

The pet-ether extract of *Mentha arvensis was* subjected to determine total reducing power. Ascorbic acid was used as reference standard (Oyaizu M, 1986).

4.2.3.1 Preparation of Standard Curve

Table 4.8: Total Reducing power of ascorbic acid

Concentration (µg/ml)	**Absorbance (at 700 nm)**	**Regression line**	**R^2 value**
250	2.657	y = 0.010x + 0.266	R^2 = 0.821
200	2.126		
150	2.284		
100	1.603		
50	0.355		

A linear relationship was observed when the absorbances were plotted against concentrations, as shown in Figure 4.6. This linear curve was considered as a standard curve.

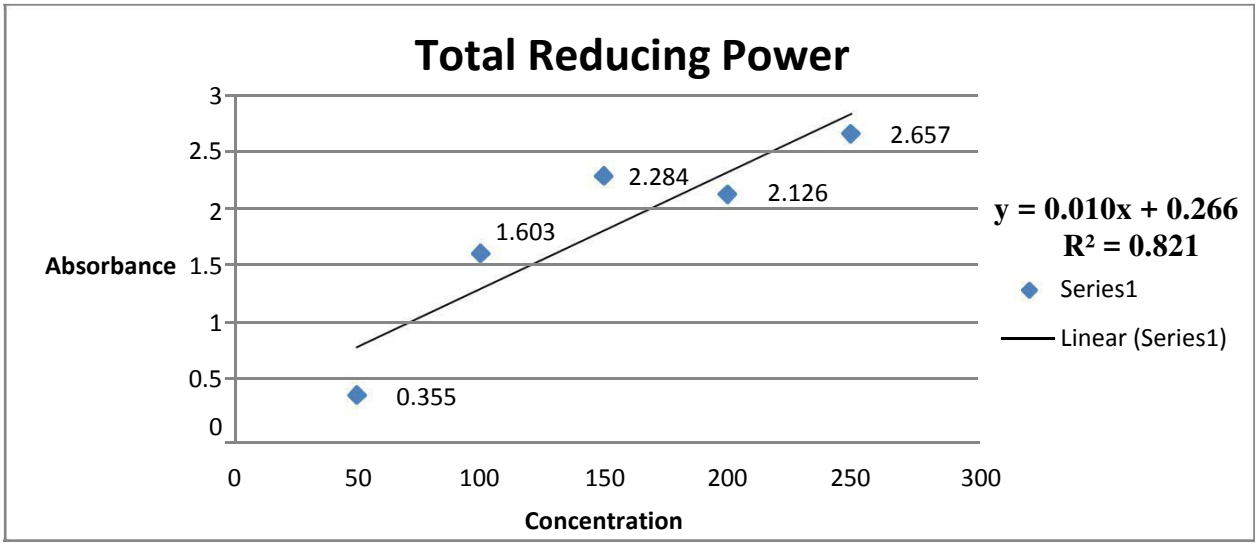


Figure 4.6: Graphical representation of reducing power of ascorbic acid

4.2.3.2 Total Reducing Power Assay in pet-ether extract of *Mentha arvensis*

Based on the absorbance values of the extract solution, reacted with potassium ferricyanide reagent and compared with the standard solutions of ascorbic acid equivalents (AAE), the total reducing power present in the extract is calculated and given in the table below.

Table 4.9: Total reducing power in pet-ether fraction of *Mentha arvensis*

Sample	**Concentration (mg/ml)**	**Absorbance (Y value at 700 nm)**	**Total reducing power (X) value (mg of AAE/gm of dried extract)**
Aqueous fraction of *Mentha arvensis*	1	0.571	27.5

4.2.3.3 Discussion

The absorbance was found to be directly proportional to the concentration. Absorbance increased with the increase in concentration indicating increase in reducing power content. Absorbance of the pet-ether fraction is less than the absorbance of standard. Based on the absorbance values of extract solution and using the regression line equation of the standard curve, 27.5 mg of AAE/gm of dried extract of reducing power content was found in the pet-ether fraction of *Mentha arvensis*

Chapter 5 : Conclusion

5.1 Conclusion

As the literature review suggests, the presence of several phytochemical compounds in pet-ether fraction of *Mentha arvensis*, makes the plant pharmacologically active.

LC_{50} value of *Mentha arvensis* in pet-ether fraction showed less cytotoxic activity than Tamoxifen. Since pet-ether fraction of *Mentha arvensis* exhibited less cytotoxic activity, so it can be investigated for anticancer, pesticidal and antitumor properties in future.

Antioxidant property in pet-ether extract of *Mentha arvensis* was determined by Phenolic content assay, Flavonoid content assay and Reducing power assay. Phenolic content was 12.315 mg/gm, Flavonoid content was 56 mg/gm and Reducing power was 27.5 mg/gm in pet-ether extract of *Mentha arvensis.*

So pet-ether extract of *Mentha arvensis* have poor antioxidant property. Mixture of compounds can lower antioxidant property in pet-ether fraction of *Mentha arvensis,* if any counteracting compounds were present in mixture. So pure compound isolation should be done in future to confirm antioxidant property of pet-ether fraction of *Mentha arvensis*.

Further investigations can be carried out to isolate and identify the active compounds present in the plant that are responsible for pharmacological activity in the development of novel and safe drugs. Other tests can be performed to evaluate some other pharmacological activities.

Chapter 6: Reference

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